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BAP1 missense mutations in cancer: friend or foe?

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Abstract

BAP1 (BRCA-associated-protein-1) is mutated in several cancers and a few therapies targeting BAP1 loss-of-function mutations have been proposed, some of them being already tested in clinical trials. However, most of missense mutations have not been functionally characterised, although such information is essential for successful patient stratification.

Background

BRCA-associated protein 1 (BAP1) gene, encoding for a deubiquitinating enzyme (DUB), is frequently altered in several tumors, including mesothelioma, uveal melanoma and renal cancer, and germline *BAP1* mutations lead to BAP1-dependent tumor predisposition syndrome (OMIM #603089) (reviewed in [1]). BAP1 and BRCA1/2, although differing in their tissue tropism, share their involvement in ubiquitin regulation and DNA damage repair [2] and show several similarities, as discussed below, in the context of the implication of their mutations in cancer.

According to COSMIC database, roughly half of *BAP1* mutations induce frameshift causing loss-of-function, and most of the remaining are missense mutations leading to amino acid (aa) changes. In this Forum, we present an overview of the current understanding of BAP1 structural domains and of the few *BAP1* missense mutations that have been functionally characterized. With this knowledge, we propose ways to prioritize the characterization of the remaining mutations in order to better understand the role of BAP1 as a cancer predisposing gene. For example, they may help understand improved survival in familial malignant mesothelioma (MM) patients bearing BAP1 mutations, relative to other MM cohorts [3].

BAP1 is a 729 aa long protein that possesses an ubiquitin carboxy-terminal hydrolase (UCH) domain at its N-terminus, followed by a short UCH37-like coiled coil motif (ULD1, also called CC1), an internal domain (ID), and at its C-terminus, a second long ULD2 (CC2), two nuclear localization signals (NLS1 and 2) and a C-terminal tail (CT, Figure 1a).

BAP1 missense mutations and their biological consequences

BAP1 plays a complex role as a transcription regulator, chromatin modulator, and, as mentioned above, has been shown to be implicated in DNA repair (reviewed in [1]). For its DUB function on chromatin, BAP1 has been suggested to form a double heterodimer [4] with

Additional Sex Comb Like (ASXL) family of epigenetic regulators [4] frequently mutated in cancer.

Unfortunately, only 38 BAP1 amino acids mutations have been functionally characterized (Supplementary Table 1) although 588 *BAP1* gene missense mutations, some of them occurring at the same residue resulting in mutations in 291 aa have been reported in COSMIC database (July 2019, Figures 1b, n=587) or classified as pathogenic in ClinVar database (n=1). UCH domain is the most frequently mutated (Figure 1c), thus potentially affecting BAP1 enzymatic activity. Therefore, functional studies have been mostly carried out on these mutations. The importance of characterizing these mutant variants is highlighted by the fact that it is difficult to predict their biological consequences. For example, BAP1 self-deubiquitination is necessary for nuclear translocation [5], therefore, mutations within UCH domain abolishing its enzymatic activity might prevent BAP1 nuclear localization and function. However, some UCH mutants with diminished enzymatic activity were described to efficiently localize to the nucleus (A95D, C91A [6], Supplementary Table 1). Sequences alignment between protein homologs from different species or between protein members of the same family, are often used to predict aa that are functionally important. Alignment of UCH, ULD1 and ULD2 domains of the human BAP1 sequence with other UCH family members, especially with UCH37 (also called UCHL5), sharing close homology with BAP1 within UCH and ULD regions, and the Drosophila ortholog of BAP1, Calypso, which allowed structural characterization [4, 7], highlights the presence of potentially pathogenic mutations affecting conserved amino acids (Figure 1d). 61 out of 113 (54%) of these conserved sites (considering only "*" and ":" marked sites) are reported as missense substitutions in COSMIC database. However, barely a quarter of them have been functionally investigated. Therefore, it is of great importance to verify all mutations predicted to have an impact on BAP1 functions, because of their possible dominant-negative action in the presence of one copy of the BAP1 wild-type (WT) allele. Indeed, several studies have documented that loss of

68 one BAP1 allele matters [8] and germline BAP1 mutations accelerate neoplastic
69 transformation in mice exposed to asbestos (reviewed in [1]). C91S mutant was shown to
70 have a dominant effect on cell growth (reviewed in [1]). C91 is in the active site (Figure 1d)
71 and tamoxifen-induction of C91A BAP1 expression in Bap1 $^{C91A/-}$ adult mice leads to the
72 same dysfunction of hematopoiesis, liver and pancreas as observed for tamoxifen-induced
73 BAP1 deficiency [9], proving the essential role of the catalytic function. Other BAP1
74 mutations (I47F, F81V, A95D) were reported to potentially cause a dominant-negative effect
75 [10]. This is likely linked to the fact that BAP1 was suggested to function as a 2:2 or 2:1
76 dimer with ASLX [4, 11] in the context of H2A deubiquitination (Figure 1e, left upper panel.
77 For matter of simplicity, only the 2:1 model is shown). This process is important for the
78 regulation of chromatin-remodeling and gene expression [12, 13]. It is yet unknown whether
79 other BAP1 functions require the same structural organisation and this model remains a
80 matter of controversy based on findings on Calypso [7], therefore, the 1:1 model is also
81 represented (Figure 1e, left lower panel). In the 2 molecules of BAP1/assembly model, in case
82 where both BAP1 WT and mutant proteins are equally abundant, one could envision a
83 scenario, in which BAP1 WT dimerizes with BAP1 mutant protein (Figure 1e, upper middle
84 panel). In this configuration, the presence of BAP1 mutant may result in the inhibition of
85 BAP1 DUB activity. Dominant-negative activity can be achieved when BAP1 mutant
86 containing dimers suppress the effect of the BAP1 WT dimers, by failing to exert
87 deubiquitination, while still capable of stable interaction with their target proteins. At least
88 one BAP1 mutant, S63C, shows an increased enzymatic activity, same ability to relocalize to
89 DNA damage site as WT, and provides survival advantage after double strand break damage
90 compared to loss-of-function UCH mutants (Supplementary Table 1). A possible gain of
91 function might therefore be expected by S63C: WT BAP1 dimer formation.
92 It is important to keep in mind that BAP1 haploinsufficiency can be triggered in a
93 physiological manner through expression of a 12 aa shorter BAP1 transcript (BAP1Δ),

missing a fragment covering parts of the UCH and ULD1 domains (missing fragment incurring in isoform BAP1Δ is represented in Figure 1a). When BAP1Δ is abundant, it may confer a dominant-negative role over the full-length BAP1 (Figure 1e, upper right panel). An expression of BAP1Δ representing at least 20% of total BAP1 leads to impaired overall BAP1 UCH activity and increased sensitivity to PARPi in mesothelioma cancer cells [14]. Interestingly, an opposite situation has been suggested for BRCA1, where high expression levels of a splice variant (BRCA1 Δ11q) confers resistance to PARPi [15], indicating that alternative splicing of both BAP1 and BRCA1 modifies therapy response.

In the 1:1 model (Figure 1e, lower middle and right panel) mutated BAP1 or BAP1Δ competition with WT BAP1 would depend on the relative amount and complex stability.

Concluding Remarks

The question arises as to whether all reported missense mutations should be functionally characterized. Characterization of *bona fide* pathogenic mutations identified in *BRCA1* or *BRCA2* tumor suppressor genes has consequences on patients handling, allowing for more precise targeting. A similar situation is likely to be true for *BAP1*. However, it would be necessary to prioritize mutations to be investigated. This would allow also identifying whether yet uncharacterized mutations act like the S63C gain-of-function mutant mentioned above. Therefore, instead of focusing on mutations occurring in conserved amino acids, one could concentrate on hot spots (here defined as >4 cases reported in COSMIC database, residues labelled in Figure 1b), where so far only 9 out of 24 have been functionally characterized. It is noteworthy that sequence alignment of UCH family does not allow predicting functional importance of ID and CT mutants, which represent 31% of missense mutations, since these domains are present only in BAP1. For this analysis alignment of BAP1 sequence from different species is necessary (Supplementary Figure 1) and reveals that

15 out of 50 conserved residues (30%) are reported as missense substitutions in COSMIC database. None of them has been functionally characterized yet.

In conclusion, a broader characterization of specific BAP1 mutations stands behind a better understanding of the biological consequences and therefore better design of the therapeutic options for patients.

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Resources

COSMIC database	https://cancer.sanger.ac.uk/cosmic
ClinVar database	https://www.ncbi.nlm.nih.gov

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Figure Legends

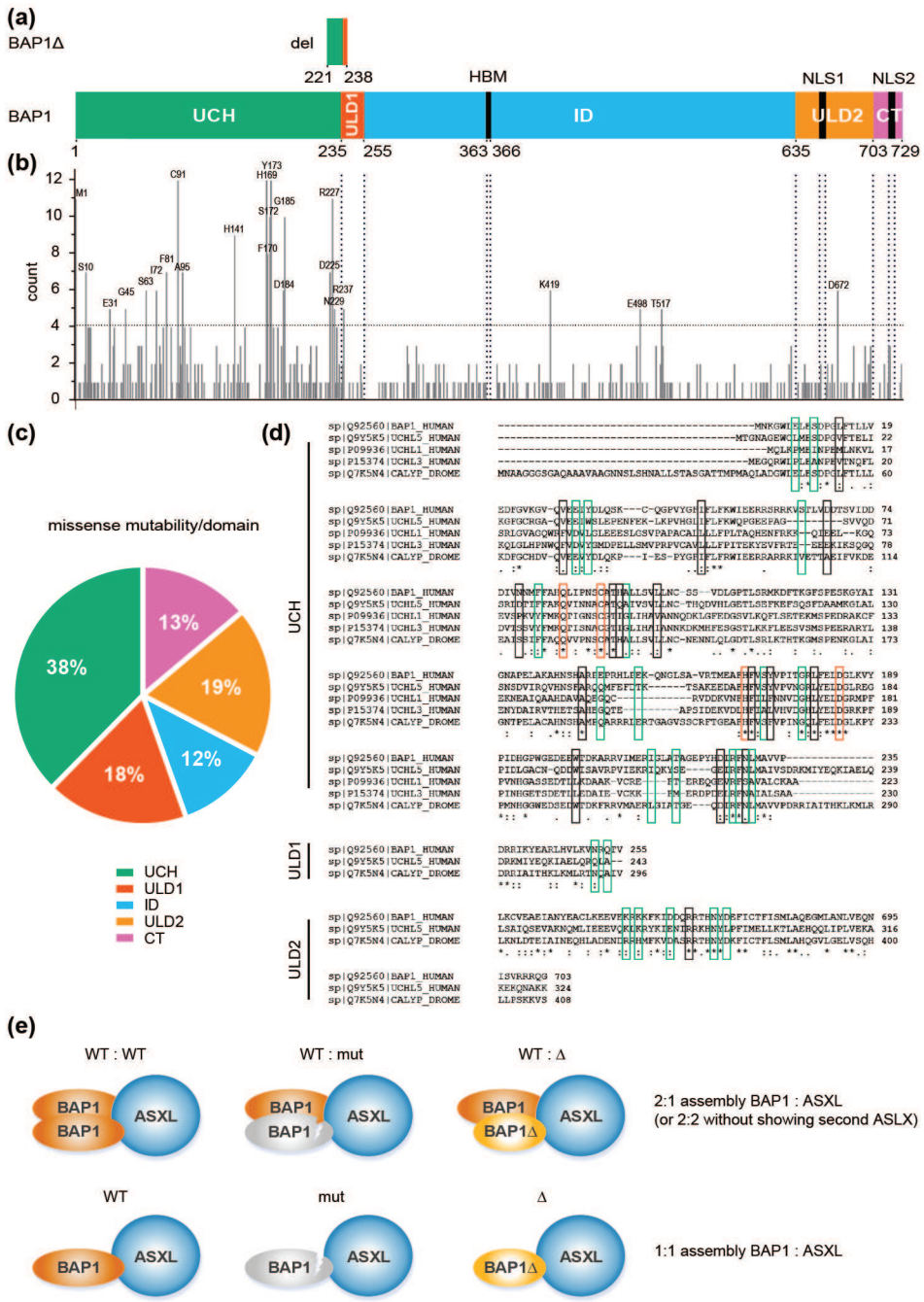

Figure 1. (a) Representation of BAP1 structure. Missing residues in isoform BAP1Δ are also represented. **(b)** Occurrence of point missense mutations within BAP1 gene listed in COSMIC database (July 2019). **(c)** Frequency of point missense mutations per residue within each single BAP1 domain, here defined as: UCH domain (1-235 aa), ULD1 (236-255 aa), ID, internal -domain (256-634 aa), ULD2 (635-703 aa) and CT (704-729 aa). Ratio between

number of mutations occurring in each of above domains and domain's length was calculated and each ratio was assessed as percentage of the sum of ratio of all domains. **(d)** Alignment of BAP1 and other UCH family members including UCHL5 (UCH37) performed by Clustal Omega online tool. Conservative sites are indicated as follows: conserved sites (*), sites with conservative replacements (:), sites with semi-conservative replacements (.). Rectangles in green highlight mutations functionally characterized in a wet-lab settings (References documented in Supplementary Table 1). In orange residues forming the catalytic site of BAP1. In black are mutations predicted to be highly pathogenic based on segregation within carriers with BAP1 tumor predisposition syndrome (References documented in Supplementary Table 1), or listed as pathogenic in ClinVar database, or inferred of being functionally important, based on structural analysis of Calypso/Asx [7]. Importantly, the mutations that are in non-conserved sequences when comparing BAP1 and other UCH family members remain nevertheless conserved when comparing all BAP1 sequences from all species. **(e)** Schematic representation of possible complex assembly of BAP1 with ASLX taking into account the presence of 2 BAP1 [4, 11] or 1 BAP1 molecule/complex [7].